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TIEN HUMAN **RETINOID** X RECEPTOR - GAMMA (hRXR-GAMMA)

AI **WO 1996-US847 A 19960119**

ABEN The present invention relates to a novel **retinoid** receptor, human **retinoid** X receptor 'gamma'. hRXR'gamma' modulates transcription of certain genes in the presence of certain **retinoid** compounds. hRXR'gamma' differs from known **retinoid** receptors in nucleotide sequence, amino acid sequence, and expression pattern in tissues. The invention provides isolated, purified, or enriched nucleic. . .

DETD . . . Probe Techniaues, p. 275 Academic Press, San Diego (Kricka, ed., 1992) hereby incorporated by reference herein in its entirety, including any drawings). **Kits** for performing such methods may be constructed to include a container means having disposed therein a nucleic acid probe.

. . . antibody under conditions such that an immunocomplex forms and detecting the presence and/or amount of the antibody conjugated to the hRXR-T polypeptide. Diagnostic **kits** for performing such methods may be constructed to include a first container means containing the antibody and a second container means having a. . .

. . . healing, including modulation of chelosis. The compounds identified herein may be used in combination with radiation therapy, chemotherapy and other biologicals such as **interferons** and interleukins.

Furthermore, one skilled in the art can readily adapt currently available procedures, as well as the techniques, methods and **kits** disclosed above with regard to antibodies, to generate peptides capable of binding to a

specific peptide sequence in order to generate. . .

. . . .

reagents which are capable of reacting with the labeled antibody. The compartmentalized kit may be as described above for nucleic acid probe **kits**. one skilled in the art will readily recognize that the antibodies described in the present invention can readily be incorporated into one. . .

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(54) Title: HUMAN RETINOID X RECEPTOR - GAMMA (hRXR-GAMMA)

(57) Abstract

The present invention relates to a novel retinoid receptor, human retinoid X receptor γ. hRXRγ modulates transcription of certain genes in the presence of certain retinoid compounds. hRXRγ differs from known retinoid receptors in nucleotide sequence, amino acid sequence, and expression pattern in tissues. The invention provides isolated, purified, or enriched nucleic acid encoding hRXRγ polypeptides and vectors containing thereof, cells transformed with such vectors, and methods of screening for compounds capable of binding hRXRγ polypeptides. The invention also provides isolated, purified, enriched, or recombinant hRXRγ polypeptides, antibodies having specific binding affinity to hRXRγ polypeptides, and hybridomas producing such antibodies.

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HUMAN RETINOID X RECEPTOR - GAMMA (hRXR-GAMMA)

Field of the Invention

This invention relates to the cloning and uses of a human retinoid X receptor subtype.

Background of the Invention

Retinoic acid is a vitamin A metabolite which has been recognized as inducing a broad spectrum of biological effects. A variety of structural analogues of retinoic acid have been synthesized that also have been found to be bioactive. Some, such as Retin-A® (registered trademark of Johnson & Johnson) and Accutane® (registered trademark of Hoffmann-LaRoche), have found utility as therapeutic agents for the treatment of various pathological conditions. Metabolites of vitamin A and their synthetic analogues are collectively herein called "retinoids". Synthetic retinoids have been found to mimic many of the pharmacological actions of retinoic acid. However, the broad spectrum of pharmacological actions of retinoic acid is not reproduced in full by all bioactive synthetic retinoids.

Medical professionals are interested in the medicinal applications of retinoids. Among their uses approved by the FDA is the treatment of severe forms of acne and psoriasis. Evidence also exists that these compounds can be used to arrest and, to an extent, reverse the effects of skin damage arising from prolonged exposure to the sun. Other evidence indicates that these compounds may be useful in the treatments of a variety of cancers including melanoma, cervical cancer, some forms of leukemia, and basal and squamous cell carcinomas. Retinoids have also been shown to be efficacious in treating premalignant cell lesions, such as oral leukoplakia, and to prevent the occurrence of malignancy.

Retinoids are able to cross passively biological membranes and control cell functions by using sp cific

intracellular receptors as signal transducers. These intracellular receptors, located in the nucleus in the presence of their retinoid ligands, function as ligand-activated transcription factors that modulate gene expression through binding to specific DNA sequences located in the regulatory regions of target genes.

Retinoids regulate the activity of two distinct intracellular receptor subfamilies; the retinoic acid receptors (RARs) and the retinoid X receptors (RXRs). The RAR and RXR subfamilies are divided into six subtypes, based upon their primary sequence homology, their ability to bind to various retinoid analogues, and by their promoter recognition sequence specificity (Mangelsdorf DJ, Umesono K, and Evans RM 1994 Retinoid receptors. In: Sporn MB, Roberts AB, and Goodman DS (eds) The Retinoids: Biology, Chemistry, and Medicine. Raven Press, pp 319-349; Giguere V, Retinoic acid receptors and cellular retinoid binding proteins: complex interplay in retinoid signalling. Endocrine Reviews 15:61-79, 1994). The RARs have three subtypes denoted α, β, and γ. The RXRs also have three known subtypes, α, β, and γ.

On one hand, RARs and RXRs share common structure and functional domains with other members of the steroid hormone receptor superfamily, comprising an amino-terminal region of variable length, a DNA-binding domain located in the central region, and a ligand-binding domain encompassing most of the carboxy-terminal end of the proteins. On the other hand, RARs and RXRs differ in several aspects. First, the RARs and RXRs are divergent in primary structure, e.g., the ligand-binding domains of RARα and RXRα have only approximately 27% amino acid identity (i.e., "homology"). These structural differences are reflected in the different relative degrees of responsiveness of RARs and RXRs to various vitamin A metabolites and synthetic retinoids.

RARs bind to both 9-cis retinoic acid (9cRA) and all-trans retinoic acid (tRA) with equally high affinity,

displaying K_d values of 0.2 - 0.8 nM (Allenby G, et al., 1993, "Retinoic acid receptors and retinoid X receptors: interactions with endogenous retinoic acids." Proc Natl Acad Sci USA 90:30-34; Allegretto EA, et al., 1993, "Transactivation properties of retinoic acid and retinoid X receptors in mammalian cells and yeast: correlation with hormone binding and effects of metabolism." J. Biol. Chem. 268:26625-26633). RXRs bind with high affinity and specificity to 9cRA (Levin AA, et al., 1992, 9-cis retinoic acid stereoisomer binds and activates the nuclear receptor RXRα. Nature 355:359-361; Heyman RA, et al., 1992, 9-cis retinoic acid is a high affinity ligand for the retinoid X receptor. Cell 68:397-406) with K_d values of 1-2 nM (Allegretto EA, et al., 1993, "Transactivation properties of retinoic acid and retinoid X receptors in mammalian cells and yeast: correlation with hormone binding and effects of metabolism." J. Biol. Chem. 268:26625-26633), but do not bind to tRA (IC_{50} > 50,000 nM versus tritiated 9cRA (Allenby G, et al., 1993, "Retinoic acid receptors and retinoid X receptors: interactions with endogenous retinoic acids." Proc. Natl. Acad. Sci. USA. 90:30-34; Allegretto EA, et al., 1993, "Transactivation properties of retinoic acid and retinoid X receptors in mammalian cells and yeast: correlation with hormone binding and effects of metabolism." J. Biol. Chem. 268:26625-26633).

In addition, distinctly different patterns of tissue distribution are seen for RARs and RXRs. For example, in contrast to the RARs, which are not expressed at high levels in the visceral tissues, RXRα mRNA has been shown to be most abundant in the liver, kidney, lung, muscle and intestine.

Furthermore, RARs and RXRs have different target gene specificity. For example, response elements in cellular retinol binding protein type II (CRBPII) and apolipoprotein AI genes confer responsiveness to RXR, but not to RAR. RAR has also been shown to repress RXR-mediated

activation through the CRBPII RXR response element (Mangelsdorf et al., Cell, 66:555-61 (1991)).

The RXR class of retinoid receptors not only function as effector molecules for 9-*cis* RA but also function as heterodimeric partners for other members of the intracellular receptor superfamily including RARs, the thyroid hormone receptor, the peroxisome proliferator-activator receptor (PPAR), the vitamin D receptor, and a number of other intracellular receptors whose ligands have not yet been identified (orphan receptors) (Leid,M., Kastner,P., Lyons,R., Nakshatri,H., Saunders,M., Zacharewski,T., Chen,J.Y., Staub,A., Garnier,J.M., Mader,S. and Chambon,P. (1992) Cell, **68**, 377-395, Yu,V.C., Delsert,C., Andersen,B., Holloway,J.M., Devary,O.V., Naar,A.M., Kim,S.Y., Boutin,J.M., Glass,C.K. and Rosenfeld,M.G. (1991) Cell, **67**, 1251-1266, Kliewer,S.A., Umesono,K., Mangelsdorf,D.J. and Evans,R.M. (1992) Nature, **355**, 446-449, Kliewer,S.A., Umesono,K., Noonan,D.J., Heyman,R.A. and Evans,R.M. (1992) Nature, **358**, 771-774, Kliewer,S.A., Umesono,K., Heyman,R.A., Mangelsdorf,D.J., Dyck,J.A. and Evans,R.M. (1992) Proc. Natl. Acad. Sci. U. S. A., **89**, 1448-1452). In fact, RXR-β was first identified in either human or rat cells biochemically by a number of laboratories using functional assays to characterize protein molecules that increased the DNA binding properties of VDR, RAR, TR, and H2BPII (Hamada,K., Gleason,S.L., Levi,B.Z., Hirschfeld,S., Appella,E. and Ozato,K. (1989) Proc. Natl. Acad. Sci. U. S. A., **86**, 8289-8293). Upon isolation and cloning of these molecules it became evident that these molecules were the human counterparts of mouse RXR-β.

Some members of the RXR family of receptors have been described in humans, rat, chicken (Rowe,A., Eager,N.S. and Brickell,P.M. (1991) Development, **111**, 771-778) and xenopus (Blumberg,B., Mangelsdorf,D.J., Dyck,J.A., Bittner,D.A., Evans,R.M. and De Robertis,E.M. (1992) Proc. Natl. Acad. Sci. U. S. A., **89**, 2321-2325). To date

only two subtypes of RXR receptors, α and β, have been characterized from humans (Mangelsdorf,D.J., Ong,E.S., Dyck,J.A. and Evans,R.M. (1990) Nature, 345, 224-229, Fleischhauer,K., Park,J.H., DiSanto,J.P., Marks,M., Ozato,K. and Yang,S.Y. (1992) Nucleic. Acids. Res., 20, 1801, Leid,M., Kastner,P., Lyons,R., Nakshatri,H., Saunders,M., Zacharewski,T., Chen,J.Y., Staub,A., Garnier,J.M., Mader,S. and Chambon,P. (1992) Cell, 68, 377-395).

Summary of the Invention

The lack of a human RXR-γ cDNA clone has hampered research such as an examination of the expression patterns of the RXR family of receptors in human tissues and cell lines. To alleviate this problem applicant cloned and characterized a human RXR-γ subtype cDNA.

The present invention relates to hRXR-γ polypeptides, nucleic acids encoding such polypeptides, cells, tissues and animals containing such polypeptides and nucleic acids, antibodies to such polypeptides, assays utilizing such polypeptides and nucleic acids, and methods relating to all of the foregoing. The hRXR-γ polypeptides, nucleic acids, and antibodies are useful for establishing the tissue specific expression pattern of hRXR-γ gene. For example, a Northern blot can be used to reveal tissue specific expression of the gene. They are also useful for screening compounds (e.g., compounds active as primary endogenous inducers of the hRXR-γ polypeptides) for improved pharmacological profiles for the treatment of diseases with higher potency, efficacy, and fewer side effects.

The present invention is based upon the identification and isolation of a novel human retinoid X receptor subtype termed hRXR-γ that is activated by binding of 9-cis retinoic acid or LG100069, i.e., (*E*)-4-[2-(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-1-propenyl]

benzoic acid. hRXR-γ has 463 amino acids and a predicted molecular weight of 55 kD.

Thus, in a first aspect the invention features an isolated, purified, enriched or recombinant nucleic acid encoding a hRXR-γ polypeptide.

By "isolated" in reference to nucleic acid is meant a polymer of 2 (preferably 21, more preferably 39, most preferably 75) or more nucleotides conjugated to each other, including DNA or RNA that is isolated from a natural source or that is synthesized. The isolated nucleic acid of the present invention is unique in the sense that it is not found in a pure or separated state in nature. Use of the term "isolated" indicates that a naturally occurring sequence has been removed from its normal cellular environment. Thus, the sequence may be in a cell-free solution or placed in a different cellular environment. The term does not imply that the sequence is the only nucleotide chain present, but does indicate that it is the predominate sequence present (at least 10 - 20% more than any other nucleotide sequence) and is essentially free (about 90 - 95% pure at least) of non-nucleotide material naturally associated with it. Therefore, the term does not encompass an isolated chromosome encoding a hRXR-γ polypeptide.

By "enriched" in reference to nucleic acid is meant that the specific DNA or RNA sequence constitutes a significantly higher fraction (2 - 5 fold) of the total DNA or RNA present in the cells or solution of interest than in normal or diseased cells or in the cells from which the sequence was taken. This could be caused by a person by preferential reduction in the amount of other DNA or RNA present, or by a preferential increase in the amount of the specific DNA or RNA sequence, or by a combination of the two. However, it should be noted that enriched does not imply that there are no oth r DNA or RNA sequences present, just that the relative amount of the sequence of interest has been significantly increased in